Cytogenetic and
Genome Research

Molecular cytogenetic characterization of pancreas cancer cell lines reveals high complexity chromosomal alterations

C.A. Griffin[a, b]   L. Morsberger[a]   A.L. Hawkins[a]   M. Haddadin[a]   A. Patel[a]
T. Ried[c]   E. Schrock[c]   E.J. Perlman[a]   E. Jaffee[b]

Departments of [a]Pathology and [b]Oncology, Johns Hopkins University, Baltimore; [c]NHGRI, Bethesda, MD (USA)

Abstract. Karyotype analysis can provide clues to significant genes involved in the genesis and growth of pancreas cancer. The genome of pancreas cancer is complex, and G-band analysis cannot resolve many of the karyotypic abnormalities seen. We studied the karyotypes of 15 recently established cell lines using molecular cytogenetic tools. Comparative genomic hybridization (CGH) analysis of all 15 lines identified genomic gains of 3q, 8q, 11q, 17q, and chromosome 20 in nine or more cell lines. CGH confirmed frequent loss of chromosome 18, 17p, 6q, and 8p. 14/15 cell lines demonstrated loss of chromosome 18q, either by loss of a copy of chromosome 18 (n = 5), all of 18q (n = 7) or portions of 18q (n = 2). Multicolor FISH (Spectral Karyotyping, or SKY) of 11 lines identified many complex structural chromosomal aberrations. 93 structurally abnormal chromosomes were evaluated, for which SKY added new information to 67. Several potentially site-specific recurrent rearrangements were observed. Chromosome region 18q11.2 was recurrently involved in nine cell lines, including formation of derivative chromosomes 18 from a t(18;22) (three cell lines), t(17;18) (two cell lines), and t(12;18), t(15;18), t(18;20), and ins(6;18) (one cell line each). To further define the breakpoints involved on chromosome 18, YACs from the 18q11.2 region, spanning approximately 8 Mb, were used to perform targeted FISH analyses of these lines. We found significant heterogeneity in the breakpoints despite their G-band similarity, including multiple independent regions of loss proximal to the already identified loss of *DPC4* at 18q21.   Copyright © 2007 S. Karger AG, Basel

Request reprints from Constance A. Griffin, MD
  Department of Pathology, Park SB202, Johns Hopkins Hospital
  600 N Wolfe St, Baltimore MD 21287 (USA)
  telephone: +1 410 955 8363; fax: +1 410 614 7440
  e-mail: cgriffin@jhmi.edu
Current address of A.L.H.: Cancer Genetics, Inc, Hackensack, NJ (USA).
Current address of M.H.: Quest Diagnostics, Teterboro, NJ (USA).
Current address of A.P.: Baylor College of Medicine, Houston, TX (USA).
Current address of E.S.: Institute for Clinical Genetics, Dresden (Germany).
Current address of E.J.P.: Children's Memorial Medical Center, Chicago, IL (USA).

Adenocarcinoma of the pancreas is a formidable foe. The disease remains silent until late in its course, rendering only about 20% of patients eligible for definitive surgical removal. The overall survival at 5 years is 5%. Many genes contribute to the pathobiology of this cancer (reviewed in Furukawa et al., 2006; Karhu et al., 2006), but the role of many genetic alterations remains to be elucidated. Cell lines continue to be an important resource for such research, especially because of the significant admixture of normal cells with tumor cells in primary specimens. We previously reported the establishment of 11 pancreatic cancer cell lines (Jaffee et al., 1998) in which allelotyping and cytogenetic analysis confirmed that the cell lines accurately reflected the primary tumors. We now report comprehensive molecular cytogenetic analysis of these and an additional four cell lines, using SKY, CGH, and YAC mapping of selected chromosomal regions.

Materials and methods

Cell lines

The methods for establishment and characterization of 11 cell lines (Panc 1.28, 2.03, 2.13, 3.27, 4.03, 4.14, 4.21, 6.03, 8.13, 9.06, 10.05) are described in detail elsewhere (Jaffee et al., 1998). Four additional cell lines from patients undergoing resection of primary adenocarcinoma of the pancreas (cell lines 2.02, 2.05, 2.08, 2.43), were established using the same conditions. All specimens were cultured in media formulated for optimal epithelial cell growth with the addition of insulin growth factors. Cell lines were grown in culture for 3–4 months and then frozen in liquid nitrogen at passage 4 to 17. When thawed for analysis, cultures were passaged an additional 4–5 times before harvest.

Karyotypes

G-band cytogenetic analysis was performed using standard techniques (Griffin et al., 1995). At least 20 metaphases were analyzed for each cell line. The G-banded karyotypes of the 11 previously described lines, with the exception of cell line 4.14, were reported previously (Jaffee et al., 1998). Karyotypes were described using guidelines established by International System for Human Cytogenetic Nomenclature (ISCN 2005).

Metaphase comparative genomic hybridization

DNA was isolated from cultured cells using standard protocols. Preparation of probes, hybridization, and image analysis was performed as previously described (Riopel et al., 1998). Briefly, reference and tumor DNA were labeled with Texas Red and FITC-dUTP (DuPont, Boston, MA) or with Spectrum-Red and Spectrum-Green-dUTP (Vysis, Downers Grove, IL) and cohybridized in equimolar amounts onto normal male metaphases. Hybridization proceeded for three days at 37°C in a moist chamber. Following washes in 2× SSC at 75°C, 37°C and room temperature, the chromosomes were counterstained with DAPI in antifade. A minimum of fifteen metaphases were captured and analyzed to generate a ratio profile. Analysis was performed utilizing dedicated software and hardware of the Cytovision system (Applied Imaging Corp., San Jose CA). Overrepresentations were interpreted from ratios >1.25; highly amplified >1.5, underrepresentations <0.75.

Spectral Karyotyping

Spectral Karyotyping ® (SKY) analysis was performed on air-dried slides, made from standard cytogenetic harvests, hybridized according to the protocol supplied by the probe manufacturer (Applied Spectral Imaging, Inc., Carlsbad, CA). Slides were incubated with the separately denatured probe mix for two days, then washed and detected. The SKY probe is a mixture of whole-chromosome paint probes for each chromosome, combinatorially labeled with five fluorochromes. Metaphase images were acquired on a Zeiss Axiophot microscope with the ASI SpectraCube SD200, and DAPI counterstained images inverted by SkyView software (ASI) to provide enhanced banding. Five cell lines were analyzed at NHGRI using a Leica microsope with SpectraCube SD200 and SkyView software. Ten metaphases were captured and analyzed for each cell line. Chromosome abnormalities were described according to ISCN (2005) guidelines whenever possible.

Targetted FISH

YACs localized to the 8p21 and 18q11.2 regions were used (Fondation Jean Dausset-CEPH, Paris, France). DNA was prepared, labeled with Spectrum Green and Spectrum Orange fluorochromes and cohybridized with CEP 8 or 18 centromere probes (Vysis) to metaphases from normal cells to verify location and lack of chimerism, and to selected cell lines using standard FISH protocols. DAPI counterstained images were used to further identify derivative and normal chromosomes.

Table 1. Summary of CGH gains and losses

Chromosome region	No. of lines (% of all lines)	Through gain/loss of entire chromosome	Through gain/loss of less than entire chromosome
GAIN			
11q	13 (86.6)	4	9
8q	11 (73.3)	3	8
20q	10 (66.6)	6	4
3q	9 (60.0)	2	7
1q	8 (53.3)	0	8
7p	7 (46.6)	2	5
7q	7 (46.6)	2	5
14q	7 (46.6)	3	4
15q	7 (46.6)	2	5
2q	6 (40.0)	3	3
12p	5 (33.3)	0	5
19q	5 (33.3)	3	2
LOSS			
18q	14 (93.3)	5	9
17p	11 (73.3)	0	11
6q	9 (60.0)	3	6
8p	9 (60.0)	0	9
10q	6 (40.0)	3	3
Y	5 (71.4)[a]	5	0

[a] Seven cell lines were from males; 5/7 = 71.4%.

Results and discussion

CGH

CGH identified extensive copy number gains and losses in all 15 cell lines, with an average of 19.5 copy changes per line. Results are summarized in Table 1 and Fig. 1. The number of genomic gains per cell line ranged from 8 to 15, with an average of 11.1.

Gain of 11q was the most common, found in 13 lines. Gain of 8q was observed in 11 lines, followed by 17q (10 lines), 20q (10 lines), 3q (9 lines), 1q (8 lines), 14q (7 lines), 15q (7 lines), 2q (6 lines), 12p (5 lines), and 19q (5 lines). Gains occurred both through gain of an entire chromosome and of a portion of a chromosome. High level gains were found most often on chromosome 8q, including 8q24.2→q24.3 in two cell lines, and at 8q21→qter and all of 8q in one line each. Other areas of high level gain were found at 3q23→qter, 14q11.2→qter, and 12p in one cell line each.

Genomic losses per cell line ranged from 2 to 15 with an average of 8.3. Loss of chromosome 18 material was the most common finding. This occurred by loss of a copy of chromosome 18 in five lines, loss of 18q in seven, and loss of a portion of 18q in two lines. Other frequent genomic losses included 17p (11 lines), 6q (nine lines), 8p (nine lines), 10q (six lines), and the Y chromosome (five of seven cell lines from males). Similar to what we observed for gains, loss occurred both through loss of an entire chromosome or portions of the chromosome.

These findings are similar to those in reports of chromosomal CGH analysis of other pancreas cancer cell lines and
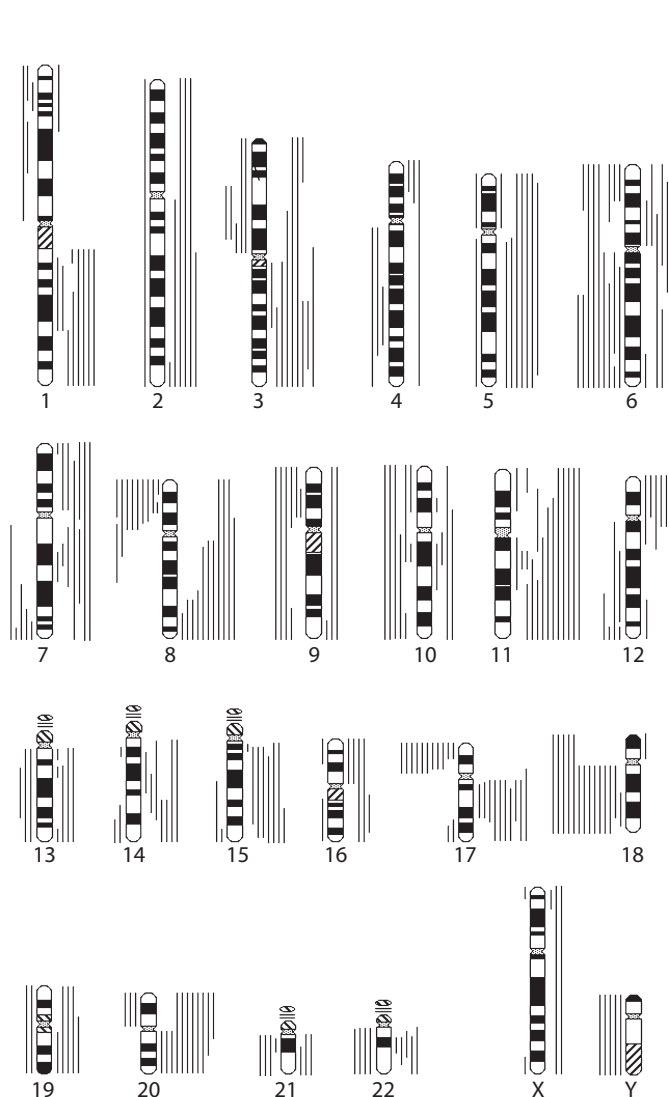
Fig. 1. Metaphase CGH analysis of 15 pancreatic carcinoma cell lines. Lines indicate areas of loss (to the left of chromosome) and gain (to the right).

Fig. 2. Chromosomal breakpoints derived by SKY and G-band karyotypes. □ SKY indicated a balanced rearrangement; ■ breakpoint derived from G-banded karyotype (SKY not performed).

primary tumors (Solinas-Toldo et al., 1996; Fukushige et al., 1997; Mahlamaki et al., 1997, 2002; Curtis et al., 1998; Ghadimi et al., 1999; Schleger et al., 2000; Shiraishi et al., 2001; Harada et al., 2002; Kitoh et al., 2005). As noted in a recent review (Karhu et al., 2006), 14–27 genetic changes per cell line were observed in published reports. As summarized in that review, the most commonly reported losses have been 6q (30–50% of cases), 9p (30–89%), 13q (15–67%), and 18q (42–89%). These series have found gains at 7q (56–67%), 8q (24–67%), 11q (56–67%), 17q (15–58%), and 20q (15–83%).

Spectral karyotyping (SKY)

The complexity of the G-band karyotypes of pancreatic cancer cell lines precludes full interpretation of structural rearrangements. We used SKY to further characterize chromosomal abnormalities in 11 lines. 93 structurally abnor-
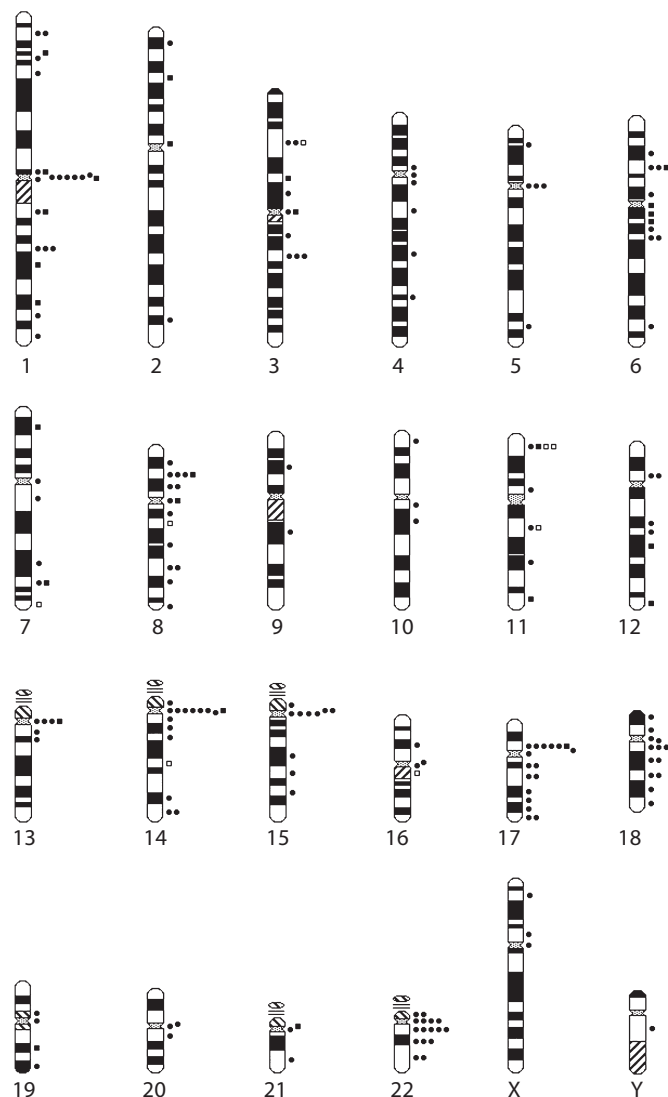
mal chromosomes were investigated, and SKY provided additional information in 67. The cell line karyotypes are listed in Table 2, and the breakpoints of clonal structural chromosomal aberrations are summarized in Fig. 2. A representative SKY metaphase is shown in Fig. 3.

Rearrangements included 11 whole arm translocations, only one of which was seen twice: der(1;7)(p10;q10) in lines 2.3 and 6.3. 14q10 was involved in translocations with 5q10, 19q10, and 22q10 one time each; 15q10 was involved in translocations with 5p10 and 20q10 one time each. Ten isochromosomes were observed: i(22)(q10) and i(13)(q10) were found in three cell lines each, while i(1)(q10), i(5)(q10), i(8)(q10), and i(14)(q10) were observed once.

Translocations that appeared to be balanced were rare. These included t(3;14)(p21;q22)del(14)(q22q32) in line 1.28, t(8;11)(q13;p15) in line 1.28, t(7;16)(q36;q11.2) in line 2.43,

Table 2. Karyotypes of pancreatic carcinoma cell lines interpreted using SKY (except Line 4.14)

Cell Line	Karyotype
Panc 6.03	70~73<4n>,XX,–Y,–Y,+der(1)t(1;20;8)(?;?;?)dup(20)(?),ins(4;1)(q26;q42q44)x2,–7,der(7)t(7;11)(q31;q22),del(8)(p21)×2, der(8)t(8;22)(p22;q13)x2,–9,–9,–10,–10,–10,der(12)t(12;18)(p11.2;q12)×2,–13,–13,der(14;19)(q10;q10), der(14;22)(q10;q10),–17,–17,–18,–18,der(18)t(18;22)(q11.2;q11.2),–19,–22,i(22)(q10),1~3dmin[cp6]
Panc 2.03	71~77<4n>,XX,der(X)t(X;1)(p22.1;q25),der(X;4)(q10;p10),del(1)(q11),der(1;3)(q10;q10)×2,+der(1;7)(p10;q10), der(5;14)(q10;q10)×2,–6,–6,dup(8)(q23q24.3)×2,der(8)t(8;18)(p21;q23)×2,–9,–9,–10,–10, der(11)t(11;17)(p15;q21)dup(11)(p14p15)×2,–13,–13,der(17)t(9;17)(q21;p11.2)×2, der(18)t(18;22)(q11.2;q11.2)dup(22)(q?)×2,der(19)t(2;19)(q36;q13.4)×2,–21,–22,–22,1~2dmin [cp5]
Panc 2.13	42~43,XX,der(1;21)(p10;p10)del(1)(p34.1p36.1),i(1)(q10),der(5;15)(p10;q10),+i(5)(q10),–6,der(6)t(3;6)(q21;p21.3), der(7)t(3;7)(q21;q32),del(8)(p21),der(9)t(9;13)(p21;q12),r(10)del(10)(?),del(16)(p12),der(17)t(17;21)(p11.2;q22), der(18)ins(6;18)(p12;p11.3q12)del(6)(p12q25),–21,–22 [cp7]/80<4n>,idemx2,–X,–X,–i(5),–i(5),–r(10),–r(10),–21,–21 [1]
Panc 9.06	70~73<4n>,XX,–X,–X,–6,–6,der(8)t(8;14)(p12;q13)×2,r(9)del(9)(?),–14,–14,der(15;20)(q10;q10)×2, der(17)t(17;22)(p11.2;q12)×2,–18,–18,der(18)t(18;22)(q11.2;q11.2),–22,–22[cp5]
Panc 8.13	48~53,X,–Y,+ider(1)(q10)t(1;8)(q21;q13),+2,+3,+del(6)(p21.3),del(8)(p12),+der(8)t(8;16)(q22;q11.1),+9,+11, +del(11)(p11.2),+12,–13,i(13)(q10),–14,ider(14)(q10)dup(14)(q11.2q31),der(15)t(14;15)(q?;p11.1)del(14)(q?)×2, +der(15)t(15;22)(p11.1;q12),–16,der(17)t(16;17)(p11.1;p11.1),+del(17)(q23),–18,+20, der(20)t(18;20)(p11.2;p11.1)×2,del(22)(q11.2)[cp4]
Panc 4.14	48~50,XX,del(6)(p21.3),+del(7)(p21),+i(8)(q10),+del(11)(p15)[cp7][a]
Panc 10.05	39,X,–Y,der(1)t(1;22)(p12;q12)ins(1;14)(p12;q32q12),–3,der(4)t(4;10)(q12;q21),der(6)t(6;17)(q14;q12)del(17)(q22q24), i(8)(q10),–10,–13,–14,–17,der(18)t(2;18)(p24;q11.2)ins(18;17)(q11.2;q12q25),–21, der(22)t(3;22)(q13.2;p11.2)t(4;22)(q31.2;q13)ins(22;1)(q13;p32p36.1),del(22)(q11.2)[cp12]
Panc 1.28	76~105<4n>,XX,–X,–X,+3,t(3;14)(p21;q22)del(14)(q22q32)×3,+5,+5,der(5)t(5;17)(p15.1;q21)×2, del(6)(p22)×2,t(8;11)(q13;p15),–9,+der(11)t(8;11)(q13;p15)×2,+12,+i(13)(q10),der(14;15)(q10;q10)×3, –17,–18,–19,+20,+20,+20,1~3 dmin[cp4]/46,XX[3]
Panc 2.05	118~123<4n>,XXXX,del(1)(q25)×2,+ider(1)(?)del(1)(?)ins(1;16)(?;?),+2,+2,del(3)(p21),+del(4)(q21), der(4)t(4;12)(p12;p11.2)×2,+der(5)t(1;5)(q25;q33)×2,+der(6)t(3;6)(q21;q15)×2,–7,+del(7)(q11.2)×2, +del(8)(q21.2)×2,dup(8)(q?),+11,+11,+del(12)(q14)×2,+13,+13,+i(13)(q10)×2,–14,+i(14)(q10)×2,+der(17;18)(p10;q10)×2, del(18)(q21)×2,+der(19)t(8;19)(q22;p12)×2,+20,+20,+20,+20,+21,+21,i(22)(q10)×2[cp5]
Panc 2.43	39~67<2n>,X,+X,der(Y)t(Y;13)(q11.2;q13),+1,+dup(1)(q12),+2,+del(3)(p12p21)x2,+4,+t(7;16)(q36;q11.2)×2, +inv(11)(q13p15),+del(15)(q21)×2,+20,+20,–22,i(22)(q10)[cp2]/91~120<4n>,idem×2[cp4]
Panc 2.02	65~71<3n>,XX,–X,–2,+5,+7,r(8)del(8)(?),–9,–10,der(10)t(8;10)(q12;p15)x2,+del(12)(q15),+der(14)t(3;14)(?;p11.2), ider(15)(q10)del(15)(q22),psu dic(15;18)(p11.2;q22)×2,der(17)t(2;17)(?;q25)del(17)(p11.2),–18, der(18)t(10;18)(q11.2;q11.1)×2,+der(20)t(8;20)(?;q11.2),+21,der(22)t(10;22)(?;p11)[cp6]
Panc 2.08	87~96,X,der(X)t(X;11)(p11.2;?),–Y,–Y,–1,der(1)t(1;14)(p11;q11.1)×2,–2,der(6)t(6;15)(q15;q24)x2,+9,+9,–10,+11, –14,–14,der(17)t(11;17)(q13;p11.2)×2,del(18)(q21)×2,+20,+20,+20,–21,–22[cp5]

[a] Karyotype established by G-banding only.

and inv(11)(q13p15) in line 2.43. Unbalanced translocations were much more common. One apparently recurrent aberration was observed, der(18)t(18;22)(q11.2;q11.2) in three cell lines. We further characterized these derivative chromosomes 18 using YACs (see below).

SKY has been utilized previously to characterize only a few pancreatic cancer cell lines. SKY analysis of cell lines AsPC1, BxPC3, Capan 2, MiaPaCa2, PANC1, and CFPAC have been studied by two groups (Ghadimi et al., 1999; Sirivatanauksorn et al., 2001). Cell lines Hs766t, A18.1, FA6, MDA Panc3, PaTu1, PaTuII, QGP1, RO, RWP, SUIT2, SW979, and T3M have been analyzed by Sirivatanauksorn et al. (2001); Capan1, and Su86.86 have been reported by Ghadimi et al. (1999). All have found multiple complexly rearranged chromosomes.

Of 144 chromosomal aberrations identified in the six cell lines that Ghadimi et al. (1999) analyzed by both SKY and CGH, only six were balanced aberrations. The only recurrently involved bands were 7q21 (in two translocations) and 7q31 (in three).

Of 344 chromosomal aberrations identified in the 20 cell lines analyzed by SKY by Sirivatanauksorn et al. (2001), 15 recurrent aberrations were found, all unbalanced. Eight of these were isochromosomes, including i(5)(p10) (in six cell lines), i(12)(p10) (n = 4), i(1)(q10) (n = 3), i(14)(q10) (n = 2), i(8)(p10) (n = 2), i(18)(p10) (n = 2), i(19)(q10) (n = 2), i(21)(q10) (n = 2), one was a Robertsonian translocation der(13;15) (q10;q10) (n = 2), and the remainder were interpreted as terminal deletions, including del(11)(q23) (n = 5), del(7)(q22) (n = 3), del(10)(p11) (n = 3), del(1)(p22) (n = 2), del(17)(q21) (n = 2), del(18)(q21) (n = 2).
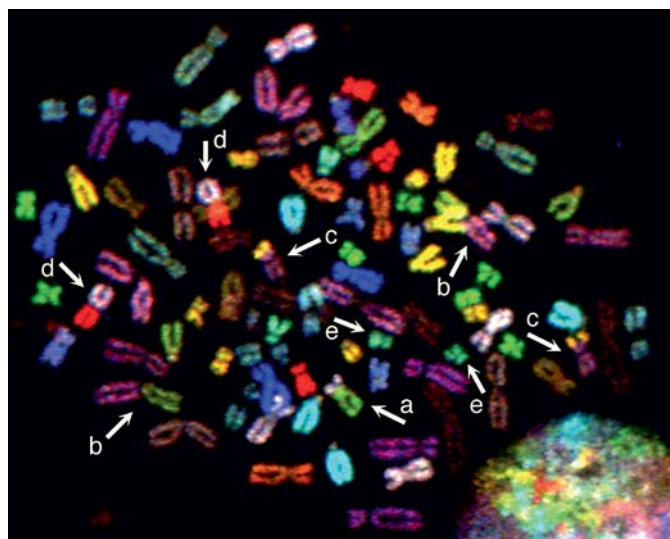
Fig. 3. SKY metaphase illustrating multiple unbalanced rearrangements in cell line 2.08. (a) der(X)t(X;11)(p11.2;?); (b) der(1)t(1;14)(p11;q11.2)×2; (c) der(6)t(6;15)(q15;q24)×2; (d) der(17)t(11;17)(q13;p11.2)×2; (e) del(18)(q21)×2.

Fig. 4. Location of the YACs used to characterize breakpoints. ISCN G-bands listed to left of the ideogram, and megabases from pter to the right. YAC locations are approximate, based on information from STS markers described in CEPH/Genathon, as located on UCSD Build March 2006. (**a**) chromosome 8; (**b**) chromosome 18.

Chromosome region 8p in pancreatic cancer

Loss of regions of the short arm of chromosome 8 is common in epithelial tumors (Birnbaum et al., 2003). Metaphase cytogenetics has identified unbalanced translocations with loss of material distal to 8p12, and CGH studies in breast and other cancers have also shown loss of distal 8p material (reviewed in Pole et al., 2006).

We found loss of 8p in nine cell lines by CGH. SKY identified seven derivative chromosomes involving 8p; these were formed as a result of an unbalanced translocation at band p21 or p22 in three lines, as an unbalanced translocation involving an unidentified portion of chromosome 8 in one line, and as an apparent terminal deletion in three lines. We used YACs to cover band 8p21 (between approximately 15 and 33 Mb from pter) (see Fig. 4a) to begin to investigate if a common breakpoint was involved. These results are summarized in Table 3. Two of the derivative chromosomes 8 retained 888d12, (the most proximal YAC at 33 Mb), while the remainder lacked signal from all four YACs tested, suggesting that the breakpoint was proximal to 888d12. Localization of the 8p breakpoints will require further mapping with additional probes.

Adelaide et al. (2003) found recurrent chromosome translocation breakpoints involving the *NRG1* gene at 8p12 in 4/34 breast and 2/9 pancreas cancer cell lines. The pancreas lines were PaTu1 and SUIT2. Recently, analysis of 8p rearrangements in 48 breast, pancreatic and colon cancer cell lines using FISH and array CGH with a tiling path of 0.2 Mb resolution over 8p12 and 1 Mb resolution over chromosome 8 was reported (Pole et al., 2006). Included in this study were nine rearrangements of 8p in seven pancreas cancer cell lines. They showed breakage and loss between 20–30 M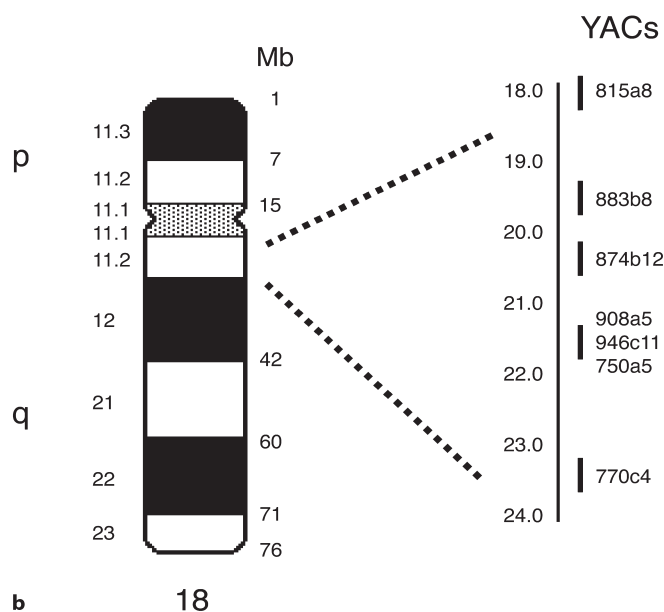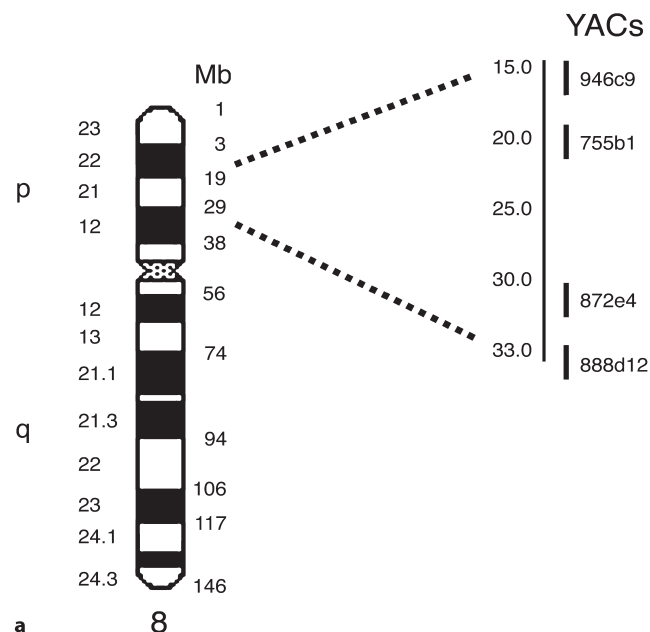b from pter in two lines, at approximately 32 Mb in two lines, between 29 and 42 Mb in two lines, and between 40–45 Mb in three lines. From their overall study, they concluded that the complexity of 8p rearrangements includes various genes proximal to 31 Mb, involving both an amplicon of *ZNF703/FLJ14299*, and *NRG1*.

Chromosome region 18q in pancreatic cancer

Monosomy of chromosome 18 has been found repeatedly in pancreas cancer. Metaphase CGH studies have

Table 3. Summary of YAC analysis of derivative chromosomes 8 in six cell lines. All YACs are located in 8p21.

Chromosome description	Panc 6.03	Panc 2.03	Panc 2.13	Panc 9.06	Panc 2.02	Panc 8.13
Partial karyotype (8s)	del(8)(p21) ×2, der(8)t(8;22)(p22;q13)	der(8)t(8;18)(p21;q23) ×2, dup(8)(q23q24.3) ×2	del(8)(p21)	der(8)t(8;14)(p12;q13) ×2	der(10)t(8;10)(q12;p15) ×2, der(20)t(8;20)(?;q11.2)	del(8)(p12), +der(8)t(8;16)(q22;q11.1)
Region of 8p loss (CGH)	8p21–pter	8p	8p21	8p	8p12–qter	8p
YAC[a]						
946c9	ND	–	–	–	ND	–
755b1	–	–	–	–	–	–
872e4	ND	ND	–	ND	–	ND
888d12	–	–	+	–	+	–

[a] + = YAC signal seen; – = no YAC signal seen on derivative chromosome; ND = not determined.

Table 4. Summary of YAC analysis of derivative chromosomes 18 in nine cell lines. All YACs are located in 18q11.2 except 881h1 in 18p11.3 (at 7 Mb).

Chromosome description	Panc 6.03	Panc 2.03	Panc 2.13	Panc 9.06	Panc 2.05	Panc 2.02	Panc 2.08	Panc 10.05	Panc 8.13
Partial karyotype (18s)	der(12)t(12;18)(p11.2;q12) ×2	der(18)t(18;22)(q11.2;q11.2) dup(22)(q?) ×2	der(18)ins(6;18)(p12;p11.3q12) del(6)(p12q25)	der(18)t(18;22)(q11.2;q11.2)	del(18)(q21) ×2 (note: FISH suggests this may be iso18p)	der(18)t(10;18)(q11.2;q11.1) ×2	del(18)(q21) ×2	der(18)t(2;18)(p24;q11.2) ins(18;17)(q11.2;q12q25)	der(20)t(18;20)(p11.2;p11.1) ×2
Region of 18 loss (CGH)	18q	18q10–q22	18q	18	18q	18	18q	18q	18q
YAC[a]									
881h1	+	+	+	–	+	–	–	+	+
815a8	+	ND	+	ND	+	+	+	+	+
883b8	+	+	+	+	–	+	+	–	+
874b12	+	+	+	+	–	+	+	–	–
762b8	+	+	+	ND	–	+	+	–	–
908a5	+	+	+	ND	+	+	–	+	–
946cl1	+	+	+	+	–	+	+	+	–
750a5	+	ND	+	ND	–	+	+	+	–
770c4	+	+	+	ND	–	+	+	–	–

[a] + = YAC signal seen; – = no YAC signal seen on derivative chromosome; ND = not determined.
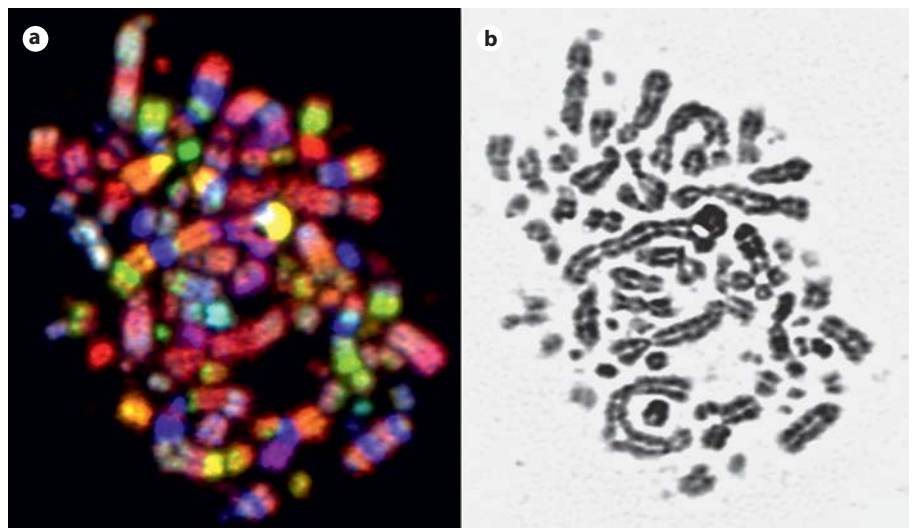
Fig. 5. The extreme complexity of a metaphase from line 2.43. (**a**) SKY, (**b**) reverse DAPI banding of same cell.

shown that partial losses of 18q are also common (Fukushige et al., 1997; Mahlamaki et al., 1997, 2002; Curtis et al., 1998; Gahdimi et al., 1999; Schleger et al., 2000; Harada et al., 2002). This has included loss of the distal half of 18q, including genes *DCC* and *SMAD4*.

Importance of 18q in the biology of pancreas cancer has been demonstrated repeatedly since early G-banded cytogenetic studies first indicated its frequent loss in this neoplasm (Johansson et al., 1992; Bardi et al., 1993; Griffin et al., 1994, 1995). Identification of *DPC4/SMAD4*, at 18q21.1 as homozygously deleted in pancreas cancer was first found in 64% of pancreas cancers studied (Hahn et al., 1996), and mutations and loss of that gene have been reported numerous times since then (reviewed in Furukawa et al., 2006). *DPC4/SMAD4* is located at 46.8 Mb in NCBI Build 36.1.

CGH analysis of the 15 cell lines reported here showed loss of chromosome 18 material to be frequent. Defining breakpoints by metaphase CGH, however, is imprecise. Our G-banding and SKY studies identified eleven derivative chromosomes 18 in nine cell lines. We used seven YACS to further investigate the possibility of involvement of a specific region in 18q11.2. These YACs map between approximately 18 and 24 Mb from 18pter (see Fig. 4b). As can be seen in Table 4, presence of specific YACs on the different derivative chromosomes 18 varied, suggesting that rearrangements in this region are complex. However, our data do not exclude the possibility of one or more specific breakpoints which might be identified using more sensitive mapping techniques in that area.

Several other investigators have described 18q breakpoint mapping in pancreas cancer. Hoglund et al. (1998) investigated breaks in 18q in 13 primary specimens of pancreatic carcinoma studied after only a few cell passages. For proximal 18q, they used FISH with YAC 766f9 (localized to 18q11.2) and a partial chromosome paint involving 18q11.1 to show that loss of 18q often was proximal to YAC 766f9. Using current databases we estimate 766f9 is located about 26.4 Mb from pter, approximately 2 Mb centromeric to the

most proximal YAC we used. Alsop et al. (2006) studied breakpoints on chromosome 18 in nine pancreas cancer cell lines using BACs from an RPC11 library. They found breakpoints in the centromere region in four lines. The breakpoint was in proximal q11 in one line, within the region bounded by BACs 296E23 and 459H24 (18.7–20.4 Mb). In two other lines, breaks were between BACs 459H24 and 5G23 (20.4–23.2 Mb). One line had breaks within 289A1 (28.3–28.5 Mb) and another between 289A1 and 459B18 (29.0–29.2 Mb). Our results, in combination with these, suggest that breakpoints within the proximal 18q region are common in pancreas cancer.

Very recently, since we completed our data, array CGH has been reported on a number of pancreas cancer cell lines (Aguirre et al., 2004; Heidenblad et al., 2004; Holzmann et al., 2004; Mahlamaki et al., 2004; Bashyam et al., 2005; Gysin et al., 2005; Nowak et al., 2005). Some of the studies have included some of the cell lines we report here. As expected, in addition to confirming regions of gain and loss identified by metaphase CGH analyses, the higher level of resolution attained by BAC and cDNA arrays has identified small regions of genomic gain and loss not previously detected. Regarding 18q, loss of both proximal and distal 18q have been found. However, none used a tiling path array on 18q. Results also emphasize those deletions which are most frequent and usually homozygous.

Aguirre et al. (2004) using a cDNA array with average coverage of 1 Mb, found deletion on 18q. The peak boundary of the most proximal deletion locus was 34.95–40.58 Mb, but is listed as extending from 18.51 to 46.28; they also detected deletion at 18q22.1→q23 from 60.4 to 77.63, peak at 74.45–76.84. Bashyam et al. (2005), using a cDNA array with average resolution of 60 kb, found the most proximal 18q deletion at 18q21 (46.7–46.8 Mb) with suggested candidate gene *WWOX*. Gysin et al. (2005) used a BAC array with average of 1.4 Mb coverage and identified loss at 18q21.1, including *DPC4*. Heidenblad et al. (2004) using cDNA and BAC arrays with 1 Mb coverage also found homozygous de-

letion in *SMAD4*. Nowak et al. (2005) used a BAC array with average 420 kb coverage; deletion of 18q11.21→q23 was found as a recurrent region of loss.

Interestingly, the 18q11 region has also been identified as amplified in two related cell lines PaTu8988T and Pa-Tu8998S in two studies. Heidenblad et al. (2004) found the amplicon at 18–20 Mb using a 1 Mb BAC array. Holzmann et al. (2004), using a 15 Mb BAC array, also found amplification in PATU 8998 at 18q11.2 and suggested *LAMA3* as a candidate gene in that amplicon. Amplification at 18q11.1→q11.2 at 16.98–18.86 Mb was found in cell line LPC6 by Heidenblad et al. (2004). This is similar to the rearrangements of 8p12 studied by Pole et al. (2006), who noted both deletions and an amplicon in 8p12.

There is reason to think that proximal 18q may harbor a tumor suppressor gene. Lefter et al. (2002) used microcell-mediated transfer of a normal copy of chromosome 18 into pancreas carcinoma cell lines. They observed suppressed growth of hybrid cells in culture and in nude mice, compared to the parental cells, regardless of the initial *DPC4/ SMAD* status of the cells, leading them to conclude that *SMAD4* was not the only tumor suppressor involved on chromosome 18. Sunamura et al. (2004) repeated this finding and used an expression array to determine that four genes related to apoptosis (not named) were upregulated in the hybrid cells.

One of the difficulties in studying pancreatic cancer cell lines is the possibility of continuing genetic instability and development of subclones. During our SKY analyses we occasionally encountered metaphases with significant genomic disarray (Fig. 5) and in evaluating 18q breakpoints we not infrequently found cells that did not contain the derivative chromosome being analyzed. Nonetheless, most described experiences with these lines have found them to be relatively stable. Indeed, of the five cell lines studied by aCGH by Nowak et al. (2005) for which we had performed metaphase CGH several years earlier, there was strong concurrence of measurement of 18q deletions.

In summary, these pancreas cancer cell lines contain significant chromosomal complexity. Data derived from CGH and SKY analyses confirm and extend the genomic gains and losses first identified by metaphase cytogenetics, and help to elucidate the chromosomal structural alterations which result in these changes.

References

Adelaide J, Huang HE, Murati A, Alsop AE, Orsetti B, et al: A recurrent chromosome translocation breakpoint in breast and pancreatic cancer cell lines targets the neuregulin/*NRG1* gene. Genes Chromosomes Cancer 37:333–345 (2003).

Aguirre AJ, Brennan C, Bailey G, Sinha R, Feng B, et al: High-resolution characterization of the pancreatic adenocarcinoma genome. Proc Natl Acad Sci USA 101:9067–9072 (2004).

Alsop AE, Teschendorff AE, Edwards PA: Distribution of breakpoints on chromosome 18 in breast, colorectal, and pancreatic carcinoma cell lines. Cancer Genet Cytogenet 164:97–109 (2006).

Bardi G, Johansson B, Pandis N, Mandahl N, Bak-Jensen E, et al: Karyotypic abnormalities in tumours of the pancreas. Br J Cancer 67:1106–1112 (1993).

Bashyam MD, Bair R, Kim YH, Wang P, Hernandez-Boussard T, et al: Array-based comparative genomic hybridization identifies localized DNA amplifications and homozygous deletions in pancreatic cancer. Neoplasia 7:556–562 (2005).

Birnbaum D, Adelaide J, Popovici C, Charafe-Jauffret E, Mozziconacci MJ, Chaffanet M: Chromosome arm 8p and cancer: a fragile hypothesis. Lancet Oncol 4:639–642 (2003).

Curtis LJ, Li Y, Gerbault-Seureau M, Kuick R, Dutrillaux AM, et al: Amplification of DNA sequences from chromosome 19q13.1 in human pancreatic cell lines. Genomics 53:42–55 (1998).

Fukushige S, Waldman FM, Kimura M, Abe T, Furukawa T, et al: Frequent gain of copy number on the long arm of chromosome 20 in human pancreatic adenocarcinoma. Genes Chromosomes Cancer 19:161–169 (1997).

Furukawa T, Sunamura M, Horii A: Molecular mechanisms of pancreatic carcinogenesis. Cancer Sci 97:1–7 (2006).

Ghadimi BM, Schrock E, Walker RL, Wangsa D, Jauho A, et al: Specific chromosomal aberrations and amplification of the *AIB1* nuclear receptor coactivator gene in pancreatic carcinomas. Am J Pathol 154:525–536 (1999).

Griffin CA, Hruban RH, Long PP, Morsberger LA, Douna-Issa F, Yeo CJ: Chromosome abnormalities in pancreatic adenocarcinoma. Genes Chromosomes Cancer 9:93–100 (1994).

Griffin CA, Hruban RH, Morsberger LA, Ellingham T, Long PP, et al: Consistent chromosome abnormalities in adenocarcinoma of the pancreas. Cancer Res 55:2394–2399 (1995).

Gysin S, Rickert P, Kastury K, McMahon M: Analysis of genomic DNA alterations and mRNA expression patterns in a panel of human pancreatic cancer cell lines. Genes Chromosomes Cancer 44:37–51 (2005).

Hahn SA, Hoque AT, Moskaluk CA, da Costa LT, Schutte M, et al: Homozygous deletion map at 18q21.1 in pancreatic cancer. Cancer Res 56:490–494 (1996).

Harada T, Okita K, Shiraishi K, Kusano N, Furuya T, et al: Detection of genetic alterations in pancreatic cancers by comparative genomic hybridization coupled with tissue microdissection and degenerate oligonucleotide primed polymerase chain reaction. Oncology 62:251–258 (2002).

Harada T, Okita K, Shiraishi K, Kusano N, Kondoh S, Sasaki K: Interglandular cytogenetic heterogeneity detected by comparative genomic hybridization in pancreatic cancer. Cancer Res 62:835–839 (2002).

Heidenblad M, Schoenmakers EF, Jonson T, Gorunova L, Veltman JA, et al: Genome-wide array-based comparative genomic hybridization reveals multiple amplification targets and novel homozygous deletions in pancreatic carcinoma cell lines. Cancer Res 64:3052–3059 (2004).

Hoglund M, Gorunova L, Jonson T, Dawiskiba S, Andren-Sandberg A, et al: Cytogenetic and FISH analyses of pancreatic carcinoma reveal breaks in 18q11 with consistent loss of 18q12–qter and frequent gain of 18p. Br J Cancer 77:1893–1899 (1998).

Holzmann K, Kohlhammer H, Schwaenen C, Wessendorf S, Kestler HA, et al: Genomic DNA-chip hybridization reveals a higher incidence of genomic amplifications in pancreatic cancer than conventional comparative genomic hybridization and leads to the identification of novel candidate genes. Cancer Res 64:4428–4433 (2004).

ISCN (2005): An International System for Human Cytogenetic Nomenclature. Shaffer LG, Tommerup N (eds) (S Karger, Basel 2005).

Jaffee EM, Schutte M, Gossett J, Morsberger LA, Adler AJ, et al: Development and characterization of a cytokine-secreting pancreatic adenocarcinoma vaccine from primary tumors for use in clinical trials. Cancer J Sci Am 4:194–203 (1998).

Johansson B, Bardi G, Heim S, Mandahl N, Mertens F, et al: Nonrandom chromosomal rearrangements in pancreatic carcinomas. Cancer 69:1674–1681 (1992).

Karhu R, Mahlamaki E, Kallioniemi A: Pancreatic adenocarcinoma – genetic portrait from chromosomes to microarrays. Genes Chromosomes Cancer 45:721–730 (2006).

Kitoh H, Ryozawa S, Harada T, Kondoh S, Furuya T, et al: Comparative genomic hybridization analysis for pancreatic cancer specimens obtained by endoscopic ultrasonography-guided fine-needle aspiration. J Gastroenterol 40:511–517 (2005).

Lefter LP, Furukawa T, Sunamura M, Duda DG, Takeda K, et al: Suppression of the tumorigenic phenotype by chromosome 18 transfer into pancreatic cancer cell lines. Genes Chromosomes Cancer 34:234–242 (2002).

Mahlamaki EH, Hoglund M, Gorunova L, Karhu R, Dawiskiba S, et al: Comparative genomic hybridization reveals frequent gains of 20q, 8q, 11q, 12p, and 17q, and losses of 18q, 9p, and 15q in pancreatic cancer. Genes Chromosomes Cancer 20:383–391 (1997).

Mahlamaki EH, Barlund M, Tanner M, Gorunova L, Hoglund M, et al: Frequent amplification of 8q24, 11q, 17q, and 20q-specific genes in pancreatic cancer. Genes Chromosomes Cancer 35: 353–358 (2002).

Mahlamaki EH, Kauraniemi P, Monni O, Wolf M, Hautaniemi S, Kallioniemi A: High-resolution genomic and expression profiling reveals 105 putative amplification target genes in pancreatic cancer. Neoplasia 6:432–439 (2004).

Nowak NJ, Gaile D, Conroy JM, McQuaid D, Cowell J, et al: Genome-wide aberrations in pancreatic adenocarcinoma. Cancer Genet Cytogenet 61:36–50 (2005).

Pole JC, Courtay-Cahen C, Garcia MJ, Blood KA, Cooke SL, et al: High-resolution analysis of chromosome rearrangements on 8p in breast, colon and pancreatic cancer reveals a complex pattern of loss, gain and translocation. Oncogene 25:5693-5706 (2006).

Riopel MA, Spellerberg A, Griffin CA, Perlman EJ: Genetic analysis of ovarian germ cell tumors by comparative genomic hybridization. Cancer Res 58:3105–3110 (1998).

Schleger C, Arens N, Zentgraf H, Bleyl U, Verbeke C: Identification of frequent chromosomal aberrations in ductal adenocarcinoma of the pancreas by comparative genomic hybridization (CGH). J Pathol 91:27–32 (2000).

Shiraishi K, Okita K, Kusano N, Harada T, Kondoh S, et al: A comparison of DNA copy number changes detected by comparative genomic hybridization in malignancies of the liver, biliary tract and pancreas. Oncology 60:151–161 (2001).

Sirivatanauksorn V, Sirivatanauksorn Y, Gorman PA, Davidson JM, Sheer D, et al: Non-random chromosomal rearrangements in pancreatic cancer cell lines identified by spectral karyotyping. Int J Cancer 91:350–358 (2001).

Solinas-Toldo S, Wallrapp C, Muller-Pillasch F, Bentz M, Gress T, Lichter P: Mapping of chromosomal imbalances in pancreatic carcinoma by comparative genomic hybridization. Cancer Res 56:3803–3807 (1996).

Sunamura M, Lefter LP, Duda DG, Morita R, Inoue H, et al: The role of chromosome 18 abnormalities in the progression of pancreatic adenocarcinoma. Pancreas 28:311–316 (2004).